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13. ABSTRACT (Maximum 200 Words)

During the progression of cancer, cells often undergo an epithelial-mesenchymal transition (EMT). This is associated with trading intercellular adhesion for a motile phenotype, as cells lose E-cadherin and gain the pro-migratory N-cadherin. While this process of cadherin switching has been described previously, the underlying molecular mechanism has yet to be elucidated. Here, we examine the role of p120 catenin (p120ctn) and the Kaiso transcription factor as mediators of cadherin switching in breast cancer. It is proposed that EMT-induced loss of E-cadherin results in the interaction of p120ctn with Kaiso transcription factor to induce expression of N-Cadherin. This is supported by the presence of putative Kaiso binding sites in the N-cadherin promoter. Moreover, p120ctn co-localizes with Kaiso in cells that have lost E-cadherin but not in those that maintain its expression. To confirm this role for p120ctn, co-immunoprecipitation of this protein with Kaiso will be utilized in an EMT model of breast cancer. Further, siRNA will be employed to demonstrate the necessity of these two proteins in EMT-induced cadherin switching. These experiments will describe a reasonable and novel mechanism for cadherin in breast cancer, while lending itself directly to the design of new therapies targeted at inhibiting breast cancer metastasis.

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Introduction

The objective of this project is to elucidate the signaling underlying an important transition in the progression of breast cancer. Intrinsic to the development of mammary carcinoma is the loss of epithelial characteristics with the acquisition of a mesenchymal-like appearance, termed the epithelial to mesenchymal transition (EMT)[1]. It has been thoroughly demonstrated that loss of the intercellular adhesion molecule E-cadherin is intrinsic to this process[2]. Moreover, in breast cancer, down-regulation of this protein is correlated with increased expression of N-cadherin, a closely related protein[3]. This process of cadherin switching has been demonstrated to be important for tumor cell survival and motility[4]. Interestingly, the mechanism by which N-cadherin is expressed upon loss of E-cadherin has not been elucidated. It was posited that p120 catenin (p120ctn), which interacts with the cytoplasmic tail of both cadherins, is released from cell junctions upon EMT-dependent down-regulation of E-cadherin and is able to enhance the transcription of the N-cadherin gene by inhibiting the Kaiso transcription repressor[5]. In addition, it was proposed that the N-cadherin promoter is also under the regulation of Twist, a transcription factor involved in metazoan development[6], and that transcription of this gene requires both the inhibition of Kaiso repression and activation by Twist.

Recent Progress

The overall goal of this project is to provide a mechanism for the switching of cadherin expression and function during the progression of breast cancer. The first aim was to demonstrate that N-cadherin is regulated by Kaiso-dependent transcriptional repression and that loss of E-cadherin results in p120ctn-mediated inhibition of this repression. To this end, I sought to develop a model of EMT in the MCF-7 human breast cancer cell line, which expresses E-cadherin and lacks N-cadherin. To generate this variant, Snail, a transcriptional repressor of E-cadherin and inducer of EMT[7], would be

stably expressed in the MCF-7 cell line. In order to accomplish this, an epitope tagged Snail construct was engineered into a retroviral expression vector, and the resulting virus was used to infect parental cells. Upon selection, however, there was no apparent change in morphology. It was determined that the Snail protein was expressed but did not induce an EMT in the MCF-7 cells. Due to this problem, it was not possible to generate a post-EMT breast cancer cell line. Interestingly, it was recently published that Snail is regulated by phosphorylation[8]. It was demonstrated that, while the wild type protein has no effect in MCF-7 cells, a mutant which cannot be phosphorylated is able to translocate into the nucleus and can induce EMT. This construct has recently been obtained and is currently being used to generate a post-EMT cell line.

Since it was proposed that p120ctn would interact with Kaiso to enhance the expression of N-cadherin, the relationship between p120ctn and Kaiso localization and cadherin expression was examined. Three human breast cancer cell lines were used for this, including MCF-7 cells that express E-cadherin but not N-cadherin, SUM-159PT cells that express N-cadherin but not E-cadherin, and MDA-MB-231 cells that express neither cadherin. It was established by immunofluorescence that Kaiso is localized to the nucleus in all cell lines, while p120ctn demonstrated nuclear localization only in those lacking E-cadherin. The catenin, however, is excluded from the nucleus of MCF-7 cells. Hence, it was concluded that p120ctn does not cause the delocalization of Kaiso in breast cancer cells upon loss of E-cadherin, but may influence Kaiso-dependent transcriptional repression within the nucleus. It still remains to be determined whether these two proteins are interacting in this cellular compartment. This work is currently under way.

p120ctn localization and ability to interact with Kaiso is proposed to be dependent upon E-cadherin expression. Therefore, the relationship between E-cadherin and N-cadherin expression is also being studied. For this, E-cadherin cDNA constructs that are either wild type, mutated for p120ctn binding or mutated for β-catenin binding[9] have been

stably introduced into SUM-159PT cells. These cells have very recently been generated and appear to grow slower than the parental cells, so it has not yet been determined whether expression of E-cadherin is influencing N-cadherin expression, regardless of catenin binding. These experiments are currently being performed.

The second aim of this project was to examine the role of the Twist transcription factor as a key regulator of N-cadherin gene expression. While little progress has been made on this, it has recently been demonstrated that Twist may be an important mediator of EMT in breast cancer and is associated with induction of EMT, including switching of cadherin expression[10]. Current experiments are aimed at expressing Twist cDNA in the MDA-MB-231 cell line to determine whether it can stimulate N-cadherin expression.

Ultimately, several technical issues have slowed the progress of this project. However, recent publications have validated either the approach or the mechanism that has been proposed, suggesting a high likelihood of success. Short term goals include generating the post-EMT MCF-7 cell line and examining it for cadherin switching and p120ctn/Kaiso colocalization and studying the SUM-159PT cells to determine whether N-cadherin expression is inhibited in a manner dependent upon p120ctn localization. Additionally, p120ctn interaction with Kaiso in the nuclei of SUM-159PT and MDA-MB-231 cells will be tested. Finally, Twist will be expressed in the MDA-MB-231 cells to establish whether this may influence N-cadherin expression. Regarding longterm objectives, the ability of Kaiso to directly interact with the N-cadherin promoter and p120ctn to regulate the migratory and survival functions of N-cadherin will be examined.

Training

In addition to the experiments above, I have also participated in the Pathobiology of Cancer workshop sponsored by the American Association for Cancer Research (AACR). This workshop provides hands-on, practical experience in histology, while also instructing

on the cause and progression of multiple types of human cancers, including those of breast, gastrointestinal, genitourinary, respiratory and hematopoietic origin. Lectures were provided in various areas of oncology, and poster sessions were offered and participated in. This was a great experience and provided a crucial understanding of this disease as it develops and presents in a clinical setting, while allowing for interaction with researchers in all areas of cancer biology. Further training included attending meetings hosted by the CNIO and Keystone Symposia. The CNIO meeting, entitled "Cadherins, Catenins and Cancer", was useful for providing an opportunity to interact with many researchers whose work is closely related to my own, and to receive advice and constructive criticism regarding this and other projects on which I am currently working. The Keystone Symposium, entitled "Development and Cancer", was also quite valuable. This meeting allowed for interaction with a very large number of researchers in multiple fields of cancer research, while hosting talks in many related fields and demonstrating a broader utility of this research. Overall, these two meetings and the AACR workshop were extremely beneficial for my training in cancer biology.

Key Research Accomplishments

- Determining the localization of p120ctn and Kaiso in three breast cancer cell lines.
- Stably expressing E-cadherin constructs in the SUM-159PT cells.
- Acquiring the appropriate reagents to generate a model of EMT in human breast cancer cells.

Reportable Outcomes

1. Abstract submitted for the Era of Hope meeting for the Department of Defense on this project, June 2005: "Mechanism of Cadherin Switching in Breast Cancer".
2. Manuscript on a related project to be submitted, May 2005: "Altered Localization of

p120ctn During EMT of Colon Carcinoma is Prognostic for Aggressive Disease"

3. Invited talk on a related project to the Biological and Biomedical Sciences graduate program annual retreat, September 2004: "Validating the Epithelial-Mesenchymal Transition (EMT) as a Model of Colon Cancer Progression".
4. Abstract and poster on related project presented at AACR Pathobiology of Cancer Workshop, July 2004: "The Role of p120ctn and Rho GTPases in the Epithelial-Mesenchymal Transition of Colon Carcinoma".
5. Abstract and poster on related project presented at Harvard Medical School, Department of Pathology annual retreat, July 2004: "The Role of p120ctn and Rho GTPases in the EMT of Colon Carcinoma".
6. Abstract and poster on related project presented at CNIO Conference, November 2004: "Altered Localization of p120ctn Occurs During the EMT of Colon Carcinoma and is Prognostic for Aggressive Disease".
7. Abstract and poster on related project presented at Keystone Symposium Cancer and Development, February 2005: "Altered Localization of p120ctn Occurs During the EMT of Colon Carcinoma and is Prognostic for Aggressive Disease".

Conclusions

During the first year of funding, progress has been made on the proposed project. Unfortunately, several problems occurred which slowed this progress somewhat, but recent advances should provide important data in the near future. Also within this time period, several training opportunities have served a valuable role in preparing me for a career in cancer biology. Finally, this predoctoral fellowship has also been accordingly cited as a source of support for multiple talks and posters presented within the past year both on this and related projects.

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